PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2004-105055
(43)Date of publication of application : 08.04.2004

(51)Int.Cl. C12N 15/09
A01K 67/027

(21)Application number : 2002-270788
(22)Date of filing : 17.09.2002

(71)Applicant : TOKAI UNIV
MIYATA TOSHIO
KUROKAWA KIYOSHI

(72)Inventor : MIYATA TOSHIO
KUROKAWA KIYOSHI
INASHIRO REIKO
NANGAKU MASAOMI
UEDA HIROHIKO
YOSHINO ATSUSHI
CHARLES VAN YPERSELE DE STRIHOU

(54) CARBONYL STRESS-RESISTANT TRANSGENIC ANIMAL

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a transgenic animal having a carbonyl stress-resistant ability.
SOLUTION: This transgenic animal is an animal transduced with a DNA encoding human glyoxalase I. The transgenic animal has the carbonyl stress-resistant ability, and is useful for elucidating the biologically adaptable mechanism to the carbonyl stress and for examining a medicine-creating design on the basis of the elucidation.

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

CLAIMS

[Claim(s)]
[Claim 1]
The carbonyl stress resistance transgenic animal which is a transgenic nonhuman animal which discovers DNA of the foreignness which carries out the code of the equivalent protein to the Homo sapiens glyoxalase I or the Homo sapiens glyoxalase I functionally.
[Claim 2]
The carbonyl stress resistance transgenic animal according to claim 1 to which DNA which carries out the code of the Homo sapiens glyoxalase I is set to array number:1 from the coding region of the base sequence of a publication.
[Claim 3]
The carbonyl stress resistance transgenic animal according to claim 1 whose nonhuman animal is a mouse.
[Claim 4]
The carbonyl stress resistance transgenic animal according to claim 1 whose nonhuman animal is Latt.
[Claim 5]
The recombination gene for carbonyl stress resistance transgenic animal production including DNA which carries out the code of the equivalent protein to the Homo sapiens glyoxalase I or the Homo sapiens glyoxalase I functionally, and the promotor who can make this DNA discover in an animal.
[Claim 6]
The production approach of a carbonyl stress resistance transgenic animal including the following process.
a) The process which introduces a recombination gene according to claim 5 into the fertilized egg of an animal
b) The process which chooses the individual holding the foreignness gene introduced among the first transgenic animals generated from the fertilized

egg of Process a
[Claim 7]
Furthermore, the production approach of a carbonyl stress resistance transgenic animal including the following processes c and d according to claim 6.
c) The process which obtains F1 animal which is made to cross the individual and normal animal which were chosen at Process b, and holds a foreignness gene by the hetero
d) The process which obtains F2 animal which is made to cross F1 animals obtained at Process c, and holds a foreignness gene by the gay

---

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]
This invention relates to the transgenic animal which has resistance from carbonyl stress.
[0002]
[Description of the Prior Art]
In in the living body, the carbonyl compound of sugar and the lipid origin is accumulated, and the condition that the protein qualification by the nonenzymatic biochemical reaction rose is called carbonyl stress (Miyata T., Kidney Int., and 55:389-399 (1999)). Relevance with adult diseases, such as aging, diabetes mellitus, or arteriosclerosis, is pointed out through the Maillard reaction [in / in a carbonyl compound / in the living body]. the nonenzymatic saccharification produced between reducing sugars, such as a glucose, and the proteinic amino group with a Maillard reaction -- it is a reaction. When Maillard (Maillard) heated the mixture of protein and reducing sugar in 1912, it reported paying attention to the phenomenon colored brown (Compt.Rend.Soc.Biol.72: Maillard L.C. and 599 (1912)). Since the Maillard reaction is participating in browning-izing produced between heat-treatment of food or storage, generation of a perfume component, taste, protein denaturation, etc., research has been advanced in the field of food chemistry.
[0003]
However, the glycosyl hemoglobin (HbA1c) which is the minute fraction of hemoglobin was identified in the living body in 1968, and it became clear that this increased in a diabetic further (Clin.Chim.Acta 22: reference 1-/Rahbar S. and 296 (1968)). The onset of adult diseases, such as diabetic complications and arteriosclerosis, or relation with progress of aging has come to attract attention from the meaning list of a Maillard reaction in the living body ignited by this. For example, it is thought that PIRARIN generated at the reaction after an AMADORI compound or the later stage product (it abbreviates to Advanced glycation end products and Following AGEs) represented by pentosidine can become the index of aging or diabetes mellitus.
[0004]
It actually sets to the patient of chronic renal failure. It is not concerned with the existence of hyperglycemia but a reactant high carbonyl compound and reactant high AGEs are being remarkably accumulated during blood and an organization (). [reference

2/Miyata T.et al.,] [Kidney Int.51:1170-1181; (1997)] 3/Miyata T.et al[of reference]., J. -- Am.Soc.Nephrol.7 : 1198-1206; (1996) 4/of reference Miyata T.et al., Kidney Int.55 : 389-399; (1999) 5/Miyata T.et al[of reference]., J.Am.Soc.Nephrol., 9 : 2349-2356 (1998). In renal failure, carbonyl stress exists and this is considered that sugar or the carbonyl compound originating in a lipid is because a lifting and protein are embellished for the proteinic amino group and a proteinic Maillard reaction (4/Miyata T.et al[of reference]., and Kidney Int.55:389-399 (1999)).

[0005]

The dialysis amyloidosis which is the complication of recent years and renal failure, The intervention of the carbonyl stress in onset progress of arteriosclerosis is reported (). [reference 6/Miyata et al.,] [J.Clin.Invest.92:1243-1252; (1993) reference 7/Miyata et] al., Proc.Natl.Acad.Sci.USA93: 2353-2358; (1996) Reference 8-/Miyata et al., FEBS Lett.437 : 24-28; (1998) 9/of reference Miyata et al., FEBS Lett.445 : The pathophysiology-meaning of the carbonyl stress in 202-206 (1999) and renal failure attracts attention.

Therefore, by removing the carbonyl compound generated in in the living body, improving a carbonyl state of stress controls generation of AGEs in renal failure, and to lead to control of progress of complication is considered by the relief pan of an organization failure.

[0006]

Furthermore, in the case of peritoneal dialysis, the wastes in blood are excreted in peritoneal dialysis fluid through the peritoneum. The peritoneal dialysis fluid (a glucose, IKODE kiss thorin, or amino acid is contained) of a hyperosmolarity has the operation which collects the reactant high carbonyl compounds accumulated into a renal failure patient's blood into intraperitoneal peritoneal dialysis fluid through the peritoneum. Therefore, the carbonyl compound concentration in peritoneal dialysis fluid rises, and the condition of carbonyl stress is brought about. Consequently, intraperitoneal protein deteriorates [the function of the peritoneum] in response to carbonyl qualification. it is thought that it participates in lowering of dewatering ability or progress of peritoneum sclerosis (10/Miyata T. et al. and 11-/[of Kidney Int.58:425-435; (2000) reference] Inagi R.et al[of reference]. --) FEBS Lett.463 : 260-264 (1999); Reference 12-/Ueda Y. et al. and Kidney Int.58:2518- 2524 and 2000; 13/Combet S.et al[of reference]., and J.Am.Soc.Nephrol.11 : 717-728 (2000).

[0007]

In addition, the thing which intraperitoneal is a carbonyl state of stress in the peritoneal dialysis patient by the glucose contained in peritoneal dialysis fluid it was proved from immunity histological examination of the peritoneum (14/Yamada K. et al. and 15-/[of Clin.Nephrol.42:354-361; (1994) reference] Nakayama M.et al[of reference]. --) Kidney Int.51 : 182-186 (1997); 10/of reference Miyata T.et al. and Kidney Int. 58 : 425-435; (2000) 11/Inagi R.et al[of reference]., and FEBS Lett.463:260-264; (1999) 13/Combet S.et al[of reference]., J.Am.Soc.Nephrol.11 : 717-728 (2000).

[0008]

The glucose in peritoneal dialysis fluid moreover, by heat sterilization To the peritoneum, a failure The various reactant carbonyl compounds to give generate (). [16/Nilsson-Thorell C/ of reference /.B.et al.,] [Perit.Dial.Int.13:208-213; (1993) reference 17/Wieslander] A. P.et al., Perit.Dial.Int., 15: 348-352; (1995) 18/Linden T.et al[of reference]., Perit.Dial.Int.18 : 290-293 (1998). The carbonyl compound generated

at this time is the methylglyoxal (MGO) and glyoxal (GO)3-deoxy glucosone (3-DG), formaldehyde, 5-hydroxymethyl furaldehyde, an acetaldehyde, or a furfural. In these, a dicarbonyl compound (GO, MGO, and 3-DG) From reactivity being high It participates in protein qualification (). [reference 19/Glomb] M. A.and Monnier V.M., J. Biol.Chem.276: 10017-10025; (1995) reference 20/Wells-Knecht KJ.et al. and Biochemistry 34: 3702-3709 (1995), it leads to the are recording to a blood vessel under the peritoneal mesothelium of AGEs (15/Nakayama M. et al. and 10-/[of Kidney Int.51:182-186; (1997) reference] Miyata T.et al[of reference]. --) Kidney Int.58 : 425-435 (2000).

[0009]

Moreover, sthenia of the blood vessel effective area of the peritoneum, a dewatering malfunction, vasodilatation, nitrogen-monoxide synthetic enzyme considered to be the factor which amplifies the vascularization, A blood vessel inner-bark growth factor (VEGF) (21/Neufeld G.et al[of reference]., and FASEB J.13:9-22 (1999)), And the Nepsilon-carboxymethyl lysine (CML) which is AGEs, (22/Ahmed M[of reference].U.et al., and J.Biol.Chem.261:4889-4894 (1986)) Colocalization-ization of pentosidine (23/Sell D[of reference].R.and Monnier V.M. and J.Biol.Chem.264:21597-21602 (1989)) and by immunity histological examination of a prolonged peritoneal dialysis patient's peritoneum It was clarified (). [reference 13/Combet] S.et al., J.Am.Soc.Nephrol.11 : 717-728; (2000) 11/Inagi R.et al[of reference]., and FEBS Letters 463: 260-264 (1999).

[0010]

Moreover, mRNA of VEGF and protein composition increase by exposing a culture peritoneal-mesothelium cell and an endothelial cell to MGO by in vitro (11/Inagi R.et al[of reference]., and FEBS Letter 463:260-264 (1999)). Furthermore, exposure to the glucose decomposition reaction nature carbonyl compound (glucose degradation reactive carbonyl compounds) of a culture mesothelial cell checks growth of a cell, viability, and inflammatory cytokine bleedoff (reference 24 / Witowski J.et al., and J.Am.Soc.Nephrol.12:2434-2441 (2001)).

[0011]

Therefore, the approach for decreasing the reactant carbonyl compound in peritoneal dialysis fluid was designed (386 reference 25-/Miyata T. et al. and Kidney Int.61:375-2002). For example, it replaces with the glucose which is the source of release of a carbonyl compound, and dialysing fluid using IKODE kiss thorin (icodextrin) and amino acid as osmotic-pressure matter is proposed. These new glucoses Dialysing fluid which is not included () [reference 26/Garcia-Lopez] E.et al. and Perit.Dial.Int. 20 (Suppl5) : S48-S56 (2000); 27/Krediet R[of reference].T.et al., Perit.Dial.Int.17 : 35-41; (1997) Reference 28-/Faller B., Kidney Int.56 : S81-S85 (1996) Carbonyl compound concentration is low (). [reference 12/Ueda] Y.et al., Kidney Int.58 : 2518-2524 (2000); 29/Schalkwijk C[of reference].G.et al., Perit.Dial.Int.20 : 796-798 (2000), It is desirable in respect of biocompatibility. In new IKODE kiss thorin and amino acid dialysing fluid, GO, MGO, 3-DG, and the total carbonyl compound level are clearly low as compared with heat sterilization glucose dialysing fluid.

[0012]

moreover Multi--compartment bag system (reference 30-/Topley N. and Periat.Dial.Int.17:42-47; (1997)) [31/Jorres A.et al/ of reference /.,] [Periat.Dial.Int.] 17 (Suppl2) : The carbonyl compound concentration of the dialysing fluid with which S42-S46 (1997) was filled up In the usual heat sterilization list, in spite of subsequent

storage It is very low (). [reference 32/Lage C.et] al. and Periat.Dial.Int. 20 (Suppl5) : S28-S32; (2000) 33/Tauer A.et al[of reference]., Biochem.Biophys.Res.Commun.280 : 1408-1414 (2001), The clinical usefulness by researchers It is checked (). [reference 34/Capelli] G.et al. and Adv.Perit.Dial. 15 : 238-242; (1999) 35/Rippe B.et al[of reference]., Kidney Int.59 : 348-357; (2001) 36/Jones S.et al[of reference]., Kidney Int.59 : 1529-2538 (2001).

[0013]

The approach using the compound with which checking AGEs formation as an approach of replacing with the dialysing fluid which does not contain a glucose is known is mentioned. Aminoguanidine (37/Brownlee M.et al[of reference]., and Science 232:1629-1632 (1986)), And OPB-9195 () [reference 9/Miyata] T.et al., FEBS Lett.445 : 202-206; (1999) 38/Miyata T.et al[of reference]., J.Am.Soc.Nephrol.11 : The compound (2000) of 1719-1725 etc. By carrying out the trap of the carbonyl compound Protein qualification It bars (9/Miyata T.et al[of reference]., and FEBS Lett.445:202-206; (1999) reference 5-/Miyata T et al., J.Am.Soc.Nephrol.9:2349-2356 (1998)). GO, MGO, and 3-DG level decrease dramatically by addition to OPB-9195 or the glucose dialysing fluid of marketing of aminoguanidine, and the pentosidine of AGEs and CML generation actually decrease (38/Miyata T.et al[of reference]., and J.Am.Soc.Nephrol.11:1719-1725 (2000)).

[0014]

Thus, the organization failure which controls carbonyl stress and originates in generation of AGEs in renal failure is mitigated, and development of various means for controlling progress of complication further is tried. And in order to promote the researches and developments about this carbonyl stress control, the model animal which has the resistance over the carbonyl stress resulting from environmental stress (a living environment, eating habits, etc.), and can consider the innovative drug development design based on a break through and it of a living body adaptation mechanism is indispensable.

[0015]

[Reference 1] Rahbar S., Clin.Chim.Acta 22: 296 (1968)
[Reference 2] Miyata T. et al., Kidney Int. 51: 1170-1181 (1997)
[Reference 3] Miyata T. et al., J.Am.Soc.Nephrol. 7: 1198-1206 (1996)
[Reference 4] Miyata T. et al., Kidney Int. 55: 389-399 (1999)
[Reference 5] Miyata T. et al., J.Am.Soc.Nephrol., 9: 2349-2356 (1998)
[Reference 6] Miyata et al., J.Clin.Invest. 92: 1243-1252 (1993)
[Reference 7] Miyata et al., Proc.Natl.Acad.Sci.USA 93: 2353-2358 (1996
[Reference 8] Miyata et al., FEBS Lett. 437: 24-28 (1998)
[Reference 9] Miyata et al., FEBS Lett. 445: 202-206 (1999)
[Reference 10] Miyata T. et al., Kidney Int. 58: 425-435 (2000)
[Reference 11] Inagi R. et al., FEBS Lett. 463: 260-264 (1999)
[Reference 12] Ueda Y. et al., Kidney Int. 58:2518-2524,2000
[Reference 13] Combet S. et al., J.Am.Soc.Nephrol. 11: 717-728 (2000)
[Reference 14] Yamada K. et al., Clin.Nephrol. 42: 354-361 (1994)
[Reference 15] Nakayama M. et al., Kidney Int. 51: 182-186 (1997)
[Reference 16] Nilsson-Thorell C.B. et al., Perit.Dial.Int. 13: 208-213 (1993)
[Reference 17] Wieslander A.P. et al., Perit.Dial.Int., 15: 348-352 (1995)
[Reference 18] Linden T. et al., Perit.Dial.Int. 18: 290-293 (1998)

[Reference 19] Glomb M.A. and Monnier V.M., J.Biol.Chem. 276: 10017-10025 (1995)
[Reference 20] Wells-Knecht K.J. et al., Biochemistry 34: 3702-3709 (1995)
[Reference 21] Neufeld G. et al., FASEB J. 13: 9-22 (1999)
[Reference 22] Ahmed M.U. et al., J.Biol.Chem. 261: 4889-4894 (1986)
[Reference 23] Sell D.R. and Monnier V.M., J.Biol.Chem. 264: 21597-21602 (1989)
[Reference 24] Witowski J. et al., J.Am.Soc.Nephrol. 12: 2434-2441 (2001
[Reference 25] Miyata T. et al., Kidney Int. 61:375-386,(2002)
[Reference 26] Garcia-Lopez E. et al., Perit.Dial.Int. 20(Suppl5): S48-S56 (2000)
[Reference 27] Krediet R.T. et al., Perit.Dial.Int. 17: 35-41 (1997)
[Reference 28] Faller B., Kidney Int. 56: S81-S85 (1996)
[Reference 29] Schalkwijk C.G. et al., Perit.Dial.Int. 20: 796-798 (2000
[Reference 30] Topley N., Periat.Dial.Int. 17: 42-47 (1997)
[Reference 31] Jorres A. et al., Periat.Dial.Int. 17(Suppl2): S42-S46 (1997)
[Reference 32] Lage C. et al., Periat.Dial.Int. 20(Suppl5): S28-S32 (2000)
[Reference 33] Tauer A. et al., Biochem.Biophys.Res.Commun. 280: 1408-1414 (2001)
[Reference 34] Capelli G. et al., Adv.Perit.Dial. 15: 238-242 (1999)
[Reference 35] Rippe B. et al., Kidney Int. 59: 348-357 (2001)
[Reference 36] Jones S. et al., Kidney Int. 59: 1529-2538 (2001)
[Reference 37] Brownlee M. et al., Science 232: 1629-1632 (1986)
[Reference 38] Miyata T. et al., J.Am.Soc.Nephrol. 11: 1719-1725 (2000)

[0016]

[Problem(s) to be Solved by the Invention]

This invention relates to the transgenic animal which has resistance from carbonyl stress.

[0017]

[Means for Solving the Problem]

this invention persons developed the carbonyl stress improvement agent which makes an active principle the enzyme equipped with glyoxalase I activity before paying attention to the detoxication system of reaction called a glyoxalase system as part of the research on control of carbonyl stress, and a carbonyl compound reducing agent (WO 01/45733). A glyoxalase system is the detoxication system of reaction which consists of two enzymes, Glyoxalase I (lactoyl GSH lyase) and Glyoxalase II (hydroxy acyl GSH hydrase). MGO which is a carbonyl compound poisonous to the living body produced by glycolytic pathway is converted into a lactic acid under existence of a glutathione (GSH) by this detoxication reaction (Douglas KT.et al. and Angew.Chem.24:31-44 (1985)).

[0018]

As an enzyme equipped with glyoxalase I activity, the enzyme of the following origins is well-known.

Mammalian tissue (546 Methods Enzymol.90,536- 541, 1982, Methods Enzymol.90,542-1982)

Yeast (30 FEBS Lett.85,275- 276, 1978, Biochem. J.183, 23- 1979)

Bacteria (999 Biochem.Biophys.Res.Commun., 141,993- 1986)

Homo sapiens (11221 J. Biol.chem. 268, 11217- 1993)

Moreover, the method of refining such glyoxalases I is also well-known.

[0019]

The glyoxalase I of the Latt liver cell origin is molecular weight 46,000, and is a dimer

which consists of a subunit of 23,000. The thing of the yeast origin consists of a monomer of molecular weight 32,000 (Marmstal et al., Biochem.J., and 183:23-30 (1979)). Although the glyoxalase I of the Pseudomonas bacillus origin is close to the thing of the animal cell origin, it is reported the monomer which consists of molecular weight 20,000 (Rhee et al. and Bioche.Biophys.Res.Commun.141:993-999 (1986)).

[0020]

Three sorts of isozymes exist in the glyoxalase I of the Homo sapiens origin (Aronsson et al. and Anal.Biochem.92:290-393 (1979)). However, there is no difference to molecular weight, amino acid composition, antigenic, etc. with these big isozymes, and it is only considered that it is that electric properties differ from it being separable with ion exchange chromatography. Therefore, these three sorts of isomers are considered to be the homodimer and heterodimer of two sorts of monomers originating in two alleles (Kompf et al. and Humangenetik 27:141-143 (1975)).

[0021]

About the glyoxalase I of the Pseudomonas bacillus origin, the gene which carries out the code of this is cloned, and all the base sequences are determined (Rhee et al. and Agric.Biol.Chem.52:2243-2246 (1988)). Moreover, the amino acid composition and gene sequence are indicated also about the Homo sapiens origin glyoxalase I (J. Biol.Chem.268:11217-11221 (1993)).

[0022]

On the other hand, the structure of the elimination and the metabolic system of a carbonyl compound in the living body is becoming clear recently. Some enzymes and the intervention of an enzyme path are reported to elimination of a carbonyl compound. An aldose reductase, aldehyde dehydrogenase, or a glyoxalase path is included in this. Activity lowering of these carbonyl compound elimination systems leads to lifting of many carbonyl compounds simultaneously. GSH and NAD (P) Redox coenzymes, such as H, are elements important for the activity of these paths (Thornalley P.J.Endocrinol Metab 3:149-166, 1996). Carbonyl compounds, such as MGO and GO, react nonenzymatic with the thiol group of GSH, and are metabolized by glyoxalase as a result. NAD(P) H activates a GSH reductase and raises GSH level. That is, a carbonyl compound elimination system is checked by lowering of GSH and NAD(P) H by the imbalance of an intracellular redox mechanism, and it leads to are recording of AGEs. A diabetic's GSH level in blood is falling and it is actually reported that the level of MGO which is a carbonyl compound is rising.

[0023]

this invention persons are MGO. Although the trap was carried out by GSH, it found out being further eliminated more promptly under glyoxalase I existence. Moreover, it checked that carbonyl compounds other than MGO in peritoneal dialysis fluid, for example, GO, and 3-DG were also promptly eliminated under GSH and glyoxalase I existence (WO 01/45733).

[0024]

Then, in developing the transgenic animal which has useful carbonyl stress resistance in examination of the innovative drug development design based on the break through and it of a living body adaptation mechanism to carbonyl stress, this invention persons paid their attention to DNA which carries out the code of this Glyoxalase I and it. this invention persons checked that the organization of the transgenic mouse produced by installation of DNA which carries out the code of the glyoxalase I eliminated various

kinds of carbonyl compounds. That is, the heart tissue of a glyoxalase I transgenic mouse reduced intentionally GO, GO in a MGO solution, and MGO concentration compared with the wild type.
A header and this invention were completed for the transgenic animal which has useful carbonyl stress resistance by installation of DNA which carries out the code of the glyoxalase I in examination of the innovative drug development design based on the break through and it of a living body adaptation mechanism to carbonyl stress being producible based on this observation result. That is, this invention relates to the following carbonyl stress resistance model animals, and the production approach of this model animal and an application. That is, this invention relates to the following carbonyl stress resistance transgenic animals.
[1] The carbonyl stress resistance transgenic animal which is a transgenic nonhuman animal which discovers DNA of the foreignness which carries out the code of the equivalent protein to the Homo sapiens glyoxalase I or the Homo sapiens glyoxalase I functionally.
[2] A carbonyl stress resistance transgenic animal given in [1] to which DNA which carries out the code of the Homo sapiens glyoxalase I is set to array number:1 from the coding region of the base sequence of a publication.
[3] A carbonyl stress resistance transgenic animal given in [1] whose a nonhuman animal is a mouse.
[4] A carbonyl stress resistance transgenic animal given in [1] whose a nonhuman animal is Latt.
[5] The recombination gene for carbonyl stress resistance transgenic animal production including DNA which carries out the code of the equivalent protein to the Homo sapiens glyoxalase I or the Homo sapiens glyoxalase I functionally, and the promotor who can make this DNA discover in an animal.
[6] The production approach of a carbonyl stress resistance transgenic animal including the following process.
a) The process which introduces the recombination gene of a publication into the fertilized egg of an animal [5]
b) The process which chooses the individual holding the foreignness gene introduced among the first transgenic animals generated from the fertilized egg of Process a
[7] The production approach of a carbonyl stress resistance transgenic animal given in [6] which furthermore includes the following processes c and d.
c) The process which obtains F1 animal which is made to cross the individual and normal animal which were chosen at Process b, and holds a foreignness gene by the hetero
d) The process which obtains F2 animal which is made to cross F1 animals obtained at Process c, and holds a foreignness gene by the gay
[0025]
In this invention, the Homo sapiens glyoxalase I is protein in which a code is carried out by DNA with the base sequence shown in array number:1. the amino acid sequence -- array number: -- it is shown in 2. The transgenic animal of this invention can introduce DNA which carries out the code of the equivalent protein not only to the Homo sapiens glyoxalase I but to the Homo sapiens glyoxalase I biologically. As such protein, the homologue of the glyoxalase I in other kinds can be shown, for example. The thing of for example, the yeast origin, Latt, and the Pseudomonas bacillus origin etc. is clarified at

the homologue of Glyoxalase I.

[0026]

Moreover, generally a polymorphism is shown that the gene of eukaryote is known for a Homo sapiens interferon gene in many cases. According to this polymorphism, even if it produces the permutation of the amino acid beyond one piece or it in an amino acid sequence, proteinic activity is usually maintained. Moreover, generally it is known for the alteration of the amino acid of one piece or some that proteinic activity will be maintained in many cases. Therefore, an array number: The gene which carries out the code of the protein which consists of an amino acid sequence which changed artificially the amino acid sequence shown in 2 can be altogether used for this invention, as long as this protein brings about carbonyl stress resistance. The glyoxalase I originating in Homo sapiens, yeast, Latt, or a mouse and its protein which has an equivalent function biologically are hereafter named generically, and it indicates as glyoxalases. In addition, even if it is the case where DNA which carries out the code of the glyoxalase I which originates in a mouse as glyoxalases is introduced into a mouse, DNA originating in the mouse introduced artificially is DNA of foreignness. However, in order to screen a compound useful to a therapy agent [in / for the transgenic animal by this invention / Homo sapiens] as a model animal of carbonyl stress resistance, it is advantageous to use DNA of the Homo sapiens glyoxalase I. It is because possibility that the effect to the Homo sapiens glyoxalase I can be reflected more faithfully is expectable in the body of a transgenic animal.

[0027]

Moreover, in consideration of the codon usage of the host who the codon to amino acid is well-known in itself, and the selection is also arbitrary, and is good, for example, uses, it can determine according to a conventional method (Grantham R.et al. and Nucleic Acids Res.9:r43 (1981)). Therefore, what changed DNA suitably is contained in DNA of this invention in consideration of the degeneracy of a codon. Furthermore, at least the section using the primer which consists of an synthetic oligonucleotide of the codon of these nucleic-acids array to which an alteration carries out the code of the desired alteration according to a conventional method can follow the specific displacement introducing method (Proc.Natl.Acad.Sci.U.S.A.81: MarkD.F.et al. and 5662) etc. in part (1984).

[0028]

Furthermore, an array number: The DNA is contained in DNA by this invention as long as the protein can hybridize with DNA which includes the base sequence of a publication in 1, and a code is carried out [protein] by the DNA brings about carbonyl stress resistance. It is thought that the array which can be hybridized in a specific array under stringent conditions has many in which a specific array has the protein which carries out a code, and similar activity. As "1xSSC, 0.1% SDS, 37-degree-C" extent and severer conditions, "0.5xSSC, 0.1% SDS, 42-degree-C" extent can be shown, and stringent conditions can usually show "0.1xSSC, 0.1% SDS, 55-degree-C" extent as still severer conditions. In addition, DNA which carries out the code of the glyoxalases in this invention can be the fragment as long as carbonyl stress resistance is brought to a transgenic animal.

[0029]

The carbonyl stress resistance transgenic animal by this invention can be obtained by the production approach of a well-known transgenic animal. A transgenic animal is

produced according to a "developmental engineering experiment manual" (the volume Tatsuji Nomura editorial supervisions and for Motonari Katsuki, Kodansha, 1989), "a new chemistry experiment lecture, an animal experiment method, etc." (the volume for Japanese biochemistry institutes, Tokyo Kagaku Dojin, 1991), etc. Below, the production protocol of a common transgenic animal is described.

[0030]

DNA which carries out the code of the glyoxalases used for creation of a transgenic animal in this invention can be obtained by the well-known approach based on the base sequence indicated on these descriptions. For example, isolation of cDNA which carries out the code of the glyoxalases is possible by screening the cDNA library of the human cell produced by well-known technique by using as a probe the synthetic oligonucleotide produced based on the DNA array shown on these descriptions. Moreover, DNA which carries out the code of the glyoxalases can be amplified by performing PCR by using this cDNA library as mold using the primer set up based on the base sequence shown in array number:1. Cloning of the magnification product is carried out based on a well-known approach.

[0031]

As for DNA which carries out the code of the glyoxalases, it is advantageous to rearrange and to consider [which connected with the promotor who can be discovered in the cell of the animal which should introduce this gene] as a gene construct. The recombination gene construct of this invention can be built DNA which carries out the code of said glyoxalases to the vector in which cloning is possible using a suitable host, and by inserting a promotor and carrying out cloning to the upstream. As a promotor who can use for this invention, the fowl beta actin promotor who can guide the manifestation of a foreign gene by broad vertebrates, such as a mouse and Latt, can be shown.

[0032]

Moreover, an enhancer is combinable in order to reinforce the manifestation of a foreign gene. For example, it is known that the enhancer originating in CMV will reinforce the manifestation of the foreign gene in mammalian.

In construction of the recombination gene construct which consists of these genes, it can have an enhancer and a promotor and the vector which has arranged the multi-cloning site for foreign gene insertion on the lower stream of a river further can be used. The vector with such structure can be built by the approach as shown in an example based on pCAGGS (Niwa H, Yamamura K and Miyazaki J (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector.Gene 108,193-200.) etc. The rabbit beta globin terminator is arranged on the lower stream of a river of a multi-cloning site, and this vector contributes to improvement in the manifestation effectiveness of the inserted foreign gene.

[0033]

With a suitable restriction enzyme, the recombination gene construct started from said vector is fully refined, and is used for creation of a transgenic animal. A transgenic animal is created by introducing said construct into the germinal cell containing an unfertilized egg, a fertilized egg, a sperm, and its progenitor cell etc. As a cell which introduces a construct, it is the phase of a single cell or an amphicytula, and the thing before 8 cell terms is usually used for the phase of the embryogenesis in generating of a nonhuman mammal, and a twist concrete target. as the introductory approach of a

construct -- a calcium phosphate method, an electric pulse method, the RIPOFE cushion method, a condensation method, a microinjection method, and party Kurgan -- law, the DEAE-dextran method, etc. are well-known. Furthermore, a transgenic animal can also be created by uniting with an above-mentioned germinal cell the transformed cell obtained in this way.

[0034]

The cell which introduces a construct can be a cell originating in all the nonhuman vertebrates that can create a transgenic animal. Specifically, cells, such as a mouse, Latt, a hamster, a guinea pig, a rabbit, a goat, a sheep, Buta, a cow, a dog, or a cat, can be used. For example, in a mouse, the fertilized egg which can introduce a construct is recoverable by making the mouse of Metz which prescribed the ovulation inducing drug for the patient cross the mouse of a normal male. Generally in a mouse fertilized egg, a construct is introduced by the microinjection to male pronucleus. What is considered that the cell which introduced the construct succeeded in installation after culture of night extent in the outside of the body is transplanted to a surrogate mother's oviduct, and a transgenic chimera animal is born. Metz which was made to cross with the male which cut the spermatic duct, and was made into the pseudopregnancy condition is used for a surrogate mother.

[0035]

The produced transgenic chimera animal is made to cross with an animal normal for birth of F1 animal after checking that the foreign gene (DNA which carries out the code of the glyoxalases) is included in the genome by analyzing the gene of the somatic cell. At this time, an individual with desirable more many copy numbers is chosen. A multiple copy is included in a part with the genome same [DNA of the foreignness generally introduced as a construct] by the serial. Usually, it is because it leads to a lot of gene expression and a clearer manifestation mold can be expected, so that there are many these inclusion copy numbers. In a somatic cell genome, it can check to a construct that the foreign gene (DNA which carries out the code of the glyoxalases) is incorporated in the direction of the right by PCR using a specific primer. Moreover, the relative comparison of a copy number is possible by dot blotting methods.

[0036]

What equips a somatic cell with a foreign gene (DNA which carries out the code of the glyoxalases) in F1 animal born as a result of this mating is the transgenic animal which can tell a foreign gene (DNA which carries out the code of the glyoxalases) to a reproductive cell with heterozygote (heterozygote). Therefore, the homozygote animal which will hold a foreign gene (DNA which carries out the code of the glyoxalases) by the gay if F2 animal which chooses what holds a foreign gene (DNA which carries out the code of the glyoxalases) to a somatic cell from F1 animals, and makes these parents can be made (homozygote)
animal is obtained as F2 animal.

[0037]

As long as DNA of the glyoxalases of foreignness is discovered, even if it is which generation of these transgenic animals, it can use for the carbonyl stress resistance transgenic animal of this invention. For example, if the glyoxalases of this foreignness are discovered even if it is the transgenic animal which holds DNA of glyoxalases by the hetero, it is useful as a carbonyl stress resistance transgenic animal.

[0038]

It can check that the transgenic animal is presenting carbonyl stress resistance by observing the following indexes. For example, the incubation of rye SETO and the carbonyl compound solution of an organ organization of a transgenic animal can be carried out by in vitro, concentration change of a carbonyl compound can be observed, and carbonyl stress resistance can be checked by the increase and decrease.

[0039]

The carbonyl compound elimination effectiveness of the carbonyl stress resistance transgenic animal of this invention can be checked by transgenic animal tissue and the incubation of a carbonyl compound solution. GO, MGO, 3DG, etc. can be made into an index as a carbonyl compound.

[0040]

These carbonyl compounds can be easily measured using reversed phase high pressure liquid chromatography (HPLC) etc., as shown in an example (Miyata T.et al. and Kidney Int.58:425-435 (2000)). Or a 2, 4-dinitrophenylhydrazine (2, 4-DNPH) can be made to be able to react with a carbonyl compound under acidity, and the coloring product to generate can also be measured with the absorbance in 360nm.

In addition, all the advanced-technology reference quoted in this description is included in this description as reference.

[0041]

The carbonyl stress resistance transgenic animal has the capacity which eliminates promptly the various carbonyl compounds which have the toxicity prescribed for the patient. Therefore, the transgenic animal of this invention is useful as a model animal which mitigates the organization failure which controls carbonyl stress and originates in generation of AGEs in renal failure, has the resistance over the carbonyl stress resulting from the environmental stress (a living environment, eating habits, etc.) for development of various means for controlling progress of complication further, and can consider the innovative drug development design based on a break through and it of a living body adaptation mechanism.

[0042]

[Example]

Hereafter, based on an example, this invention is explained still more concretely.

[Example 1] recombination gene construct

The Homo sapiens glyoxalase I cDNA is a carrier beam about supply from the Sagami Chuo Kagaku lab (Kanagawa, Japan). In order to build a Homo sapiens glyoxalase I cDNA installation gene construct, DNA Ligation Kit Ver.2 (TAKARA SHUZO) were used and cloning of this cDNA was carried out to the EcoRI site of pBsCAG-2. In addition, nest production of pBsCAG-2 was carried out to the SalI-PstI part of pBluescript II SK (-) and (Invitrogene) in the SalI-PstI fragment of pCAGGS (it has Niwa H et al., Gene 108:193-200 (1991), a CMV enhancer, a fowl beta actin promotor, and a rabbit beta globin terminator). Restriction enzymes Kpn I and Sac I cut pBsCAG-2 containing Glyoxalase I, the fragment containing Glyoxalase I cDNA was collected and refined, and it was used for production of a transgenic mouse (drawing 1).

[0043]

Production of a [example 2] transgenic mouse

Abdominal administration of the PMSG (pregnant-mare-serum Gonatrophin) was carried out to the female mouse (C57BL/6) of eight to 20-week ** in the three days before evening of injection, and abdominal administration of the hCG (Homo sapiens

placenta nature Gonatrophin) was carried out in the evening the two days after. Following this, it put one male mouse (C57BL/6) of 8 -20-week ** at a time into the gage, and mating was started. The vaginal plug was inspected during the morning of the next day of mating, and the female mouse which has checked the vaginal plug was moved to the Whitten culture medium which isolated and carried out HIARURONITAZE addition of the uterine tube after slaughter by cervical-vertebra dislocation. The egg was made to discharge under a stereoscopic microscope and separation and washing of a fertilized egg were performed.

[0044]

Five to 30 fertilized eggs were moved to the inverted microscope with differential interference equipment (NOMARU skiing equipment) at the culture medium drop on a hole vacancy slide glass using the system which combined the manipulator, and the microinjection of the DNA solution of 2 pL containing the DNA fragment prepared by the above of about 2,000 copies per fertilized egg was carried out to male pronucleus. The egg which DNA impregnation ended was cultivated until it transplanted to the oviduct. Transplantation parts differed in the developmental stage of a germ, the germ of a 1-2 cell term was transplanted into the oviduct, and the germ of a 8 cell term-blastocyst term was transplanted into the uterus.

[0045]

In advance of embryo transfer actuation, activation of the corpus luteum of a recipient scalpel was performed and induction of the pseudopregnancy was carried out. That is, infertile copulation was carried out to the male which carried out vasoligature of Metz of a proestrus. The delivery scheduled day of a transplantation germ made the 1st day the day which the vaginal plug of a recipient scalpel attached, and calculated it as the 20th day. When transplanting into an oviduct, the germ of 1 cell term and 2 cell terms was transplanted under the stereoscopic microscope in the oviduct of the recipient mouse of a pseudopregnancy day eye under the Nembutal anesthesia. About ten germs per single-sided oviduct were transplanted. When transplanting in a uterus, the germ which made it generate from a morula term by explantation at a blastocyst term was transplanted in the uterus of the recipient mouse which carried out induction of the pseudopregnancy under the Nembutal anesthesia. The pseudopregnancy age in day of a recipient mouse was calculated more youthfully [for one day] than the age in day of a germ. the case where judged the number of fetuses from appearance and the number of fetuses is expected to be five or more animals -- a natural birth -- the cesarean section was performed when four or less animals became. The produced mouse was separated from parents between three to four weeks of after the birth, and divided and bred the sex.

[0046]

Selection of a [example 3] transgenic mouse

A part of tail was cut after four week ** of after the birth, and genomic DNA was extracted using the kit (Qiagen DNeasy tissue kit; Qiagen). This was made into mold and magnification by PCR of an introductory gene fragment was performed. In magnification Cytomegalovirus enhancer primer (drawing 1) [Pr] 1) (sense:) [5'-GTC GAC ATT] GAT TAT TGA CTA G-3' / array number : 3, and antisense:5'-CCA TAA GGT CAT GTA CTG-3'/array number:4 and 3 of Homo sapiens glyoxalase I gene and vector'-junction The primer for the included fragment (drawing 1) [Pr] Antisense:5 '-TCGAGG GAT CTT CAT AAG AGA AGA G -3' / 5 and array number:6) is used. 2) --

(-- sense: -- 5 -- '-GTA GTG TGG GTG ACT CCT CCG TTC CTT GGG -3' -- /array number: -- The individual from which the magnification product by PCR is acquired was sorted out. Obtained F zero-generation six individuals (male 3 individual, female 3 individual) were made to cross with a normal individual (C57BL/6N Jcl), and F1 generation was obtained.

[0047]

The animal was bred in the metabolic turnover cage under the condition of free intake of water. The blood sample extracted the twenty-four-hours urine for measuring the amount of urinary protein one day before slaughter at the time of slaughter. The sample of an organ was fixed with 4% of neutral buffer formaldehyde, carried out paraffin embedding and gave hematoxylin and eosin (HE) or PAS stain after judging to 4 micrometers. a wild type or hetero glyoxalase the organization (50mg) of I TRANS GENIC F1 mouse homogenizes by NaPB (pH7.0) of 1mL containing 0.02% of Triton-X -- having -- 4 degrees C -- 20,000gx -- glyoxalase carry out centrifugal separation for 20 minutes, and according supernatant liquid to an immuno blot I analysis and glyoxalase It was used as an organization extract for assessment of I activity and carbonyl compound control.

[0048]

[Example 4] immuno blot analysis

Homo sapiens glyoxalase by which the high manifestation was carried out by the transgenic mouse It is anti-Homo sapiens glyoxalase about I gene product (about 24 kDa). It checked by immuno blot analysis of the organization homogenate using I antibody. The protein of 30microg extracted from the organization was boiled for 5 minutes, and it denaturalized, and imprinted to the polyvinylidene difluoride (PVDF) membrane (Bio Rad Lab.) after separation by SDS-PAGE using an acrylamide gradient gel (4-20%). Rabbit anti-Homo sapiens glyoxalase after blocking a membrane with the Tris buffer solution (TBS) containing 0.05% of Tween 20, and 2% of bovine serum albumin at 4 degrees C overnight I IgG (1microg/mL) (Ranganathan S et al., Biochem.Biophys.Acta., and 1182:311-316 (1993)) was incubated at 4 degrees C overnight. It incubated after washing in TBS containing 0.05% of Tween 20 with the alkaline phosphatase indicator goat origin anti-rabbit IgG F(ab') 2 (Cappel), and the membrane was colored using the nitroblue tetrazolium and X phosphoric-acid solution (Bio Rad Lab.). The anti-actin antibody (Sigma) was used for scaling, using 10% of erythrocyte suspension as contrast. A result is shown in drawing 2 . Homo sapiens glyoxalase The kidney and heart tissue which were extracted from I transgenic mouse compare with a wild type, and are high glyoxalase intentionally. I manifestation was shown (lanes 4, 5, 7, and 8). Moreover, glyoxalase The manifestation of I was increasing in all the organizations that inspected.

[0049]

Glyoxalase under [example 5] transgenic-mouse organization I activity

Glyoxalase under transgenic-mouse organization I activity was measured at 25 degrees C by detection (McLellan Aet al. and Mech Ageing Dev.48:63-71 (1989)) of an increment with an absorbance [by generation of the S-D-lactoyl GSH for 2 minutes] of 240nm. A result is shown in a table 1. Glyoxalase of the heart obtained from two sorts of lines of a transgenic mouse, liver, and the protein extract of kidney tissue I activity was intentionally high compared with the wild type.

[0050]

[A table 1]

	心臓t (Unit/g)	肝臓 (Unit/g)	腎臓 (Unit/g)	赤血球 (mUnit/ml)	血漿 (mUnit/ml)
グリオキサラーゼ I Tg/+ ライン A	1096.2±134.5	237.6±51.0	242.8±10.4	0.73±0.14	0.06±0.01
グリオキサラーゼ I Tg/+ ライン B	719.4±55.3	241.0±61.6	84.0±34.3	0.66±0.15	0.49±0.22
野生型	20.1±4.1	169.5±52.1	37.2±2.4	0.69±0.07	0.05±0.05

[0051]
Carbonyl compound elimination ability of a [example 6] transgenic-mouse organization Glyoxalase 37 degrees C incubated the heart origin protein extract, GO, or the MGO solution (100microM) of I transgenic mouse (line A) for 1 hour, and change of GO and MGO concentration was measured using reversed phase high pressure liquid chromatography (HPLC) (Miyata T.et al. and J.Am.Soc.Nephrol.11:1719-1725 (2000)). A result is shown in drawing 3 . The heart tissue extract of the transgenic-mouse origin decreased GO and MGO concentration intentionally compared with the wild type. Moreover, this lowering was dependent on the amount of added GSH.
[0052]
[Example 7] transgenic rat
It applies to an example 1 thru/or the publication of 3 correspondingly, and is glyoxalase. I transgenic rat was produced. Glyoxalase in the protein extract of the heart of the transgenic rat obtained according to the experiment approach of an example 4 and an example 5, the kidney, and antinode membrane I manifestation was checked. A result is shown in drawing 4 thru/or drawing 6 . It also sets in which organization and is glyoxalase. I activity was intentionally high compared with the wild type.
Moreover, according to the experiment approach of an example 6, the carbonyl compound elimination ability of transgenic rat heart tissue was examined. A result is shown in drawing 7 and drawing 8. The heart tissue protein extract of the transgenic rat origin decreased intentionally GO (drawing 7 A) and MGO concentration (drawing 7 B) compared with the wild type. Moreover, this lowering was dependent on the amount of added GSH.
[0053]
[Effect of the Invention]
It is Homo sapiens glyoxalase by this invention. The carbonyl stress resistance transgenic animal by installation of I gene was offered. The innovative drug development design based on the break through and it of a living body adaptation mechanism to carbonyl stress is attained using the transgenic animal of this invention.
[0054]
[Layout Table]

SEQUENCE LISTING

(110) Tokai University Educational System
Miyata, Toshio
Kurokawa, Kiyoshi

(120) Carbonyl stress resistant transgenic animal

(130) KRK-X0206

(140)
(141)

(160) 6

(170) PatentIn Ver. 2.1

(210) 1
(211) 552
(212) DNA
(213) Homo sapiens

(220)
(221) CDS
(222) (1)..(552)

(400) 1

```
atg gca gaa ccg cag ccc ccg tcc ggc ggc ctc acg gac gag gcc gcc   48
Met Ala Glu Pro Gln Pro Pro Ser Gly Gly Leu Thr Asp Glu Ala Ala
 1               5                  10                  15
```

```
ctc agt tgc tgc tcc gac gcg gac ccc act acc aag gat ttt cta ttg   96
Leu Ser Cys Cys Ser Asp Ala Asp Pro Thr Thr Lys Asp Phe Leu Leu
            20                  25                  30

cag cag acc atg cta cga gtg aag gat cct aag aag tca ctg gat ttt  144
Gln Gln Thr Met Leu Arg Val Lys Asp Pro Lys Lys Ser Leu Asp Phe
        35                  40                  45

tat act aga gtt ctt gga atg acg cta atc caa aaa tgt gat ttt ccc  192
Tyr Thr Arg Val Leu Gly Met Thr Leu Ile Gln Lys Cys Asp Phe Pro
    50                  55                  60

att atg aag ttt tca ctc tac ttc ttg gct tat gag gat aaa aat gac  240
Ile Met Lys Phe Ser Leu Tyr Phe Leu Ala Tyr Glu Asp Lys Asn Asp
65                  70                  75                  80

atc cct aaa gaa aaa gat gaa aaa ata gcc tgg gcg ctc tcc aga aaa  288
Ile Pro Lys Glu Lys Asp Glu Lys Ile Ala Trp Ala Leu Ser Arg Lys
                85                  90                  95

gct aca ctt gag ctg aca cac aat tgg ggc act gaa gat gat gcg acc  336
Ala Thr Leu Glu Leu Thr His Asn Trp Gly Thr Glu Asp Asp Ala Thr
            100                 105                 110

cag agt tac cac aat ggc aat tca gac cct cga gga ttc ggt cat att  384
Gln Ser Tyr His Asn Gly Asn Ser Asp Pro Arg Gly Phe Gly His Ile
        115                 120                 125
```

gga att gct gtt cct gat gta tac agt gct tgt aaa agg ttt gaa gaa 432
Gly Ile Ala Val Pro Asp Val Tyr Ser Ala Cys Lys Arg Phe Glu Glu
130 135 140

ctg gga gtc aaa ttt gtg aag aaa cct gat gat ggt aaa atg aaa ggc 480
Leu Gly Val Lys Phe Val Lys Lys Pro Asp Asp Gly Lys Met Lys Gly
145 150 155 160

ctg gca ttt att caa gat cct gat ggc tac tgg att gaa att ttg aat 528
Leu Ala Phe Ile Gln Asp Pro Asp Gly Tyr Trp Ile Glu Ile Leu Asn
165 170 175

cct aac aaa atg gca acc tta atg 552
Pro Asn Lys Met Ala Thr Leu Met
180

〈210〉 2
〈211〉 184
〈212〉 PRT
〈213〉 Homo sapiens

〈400〉 2
Met Ala Glu Pro Gln Pro Pro Ser Gly Gly Leu Thr Asp Glu Ala Ala
1 5 10 15

Leu Ser Cys Cys Ser Asp Ala Asp Pro Thr Thr Lys Asp Phe Leu Leu
20 25 30

Glu Gln Thr Met Leu Arg Val Lys Asp Pro Lys Lys Ser Leu Asp Phe

35 40 45

Tyr Thr Arg Val Leu Gly Met Thr Leu Ile Gln Lys Cys Asp Phe Pro
50 55 60

Ile Met Lys Phe Ser Leu Tyr Phe Leu Ala Tyr Glu Asp Lys Asn Asp
65 70 75 80

Ile Pro Lys Glu Lys Asp Glu Lys Ile Ala Trp Ala Leu Ser Arg Lys
85 90 95

Ala Thr Leu Glu Leu Thr His Asn Trp Gly Thr Glu Asp Asp Ala Thr
100 105 110

Gln Ser Tyr His Asn Gly Asn Ser Asp Pro Arg Gly Phe Gly His Ile
115 120 125

Gly Ile Ala Val Pro Asp Val Tyr Ser Ala Cys Lys Arg Phe Glu Glu
130 135 140

Leu Gly Val Lys Phe Val Lys Lys Pro Asp Asp Gly Lys Met Lys Gly
145 150 155 160

Leu Ala Phe Ile Gln Asp Pro Asp Gly Tyr Trp Ile Glu Ile Leu Asn
165 170 175

Pro Asn Lys Met Ala Thr Leu Met
180

〈210〉 3
〈211〉 22
〈212〉 DNA
〈213〉 Artificial Sequence

〈220〉
〈223〉 Description of Artificial Sequence:an artificially synthesized primer sequence

〈400〉 3
gtcgacattg attattgact ag 22

〈210〉 4
〈211〉 18
〈212〉 DNA
〈213〉 Artificial Sequence

〈220〉
〈223〉 Description of Artificial Sequence:an artificially synthesized primer sequence

〈400〉 4
ccataaggtc atgtactg 18

〈210〉 5
〈211〉 30
〈212〉 DNA

〈213〉 Artificial Sequence

〈220〉

〈223〉 Description of Artificial Sequence:an artificially synthesized primer sequence

〈400〉 5

gtagtgtggg tgactcctcc gttccttggg 30

〈210〉 6

〈211〉 25

〈212〉 DNA

〈213〉 Artificial Sequence

〈220〉

〈223〉 Description of Artificial Sequence:an artificially synthesized primer sequence

〈400〉 6

tcgagggatc ttcataagag aagag 25

[0055]

[Brief Description of the Drawings]

[Drawing 1] Glyoxalase Drawing showing the structure of the DNA fragment used for the microinjection to the egg which started and was extracted from I installation gene construct.

[Drawing 2] Glyoxalase Drawing showing immuno blot analysis of I gene product. Rain 1:10% mouse erythrocyte suspension (contrast), rain 2:10% human-erythrocyte suspension (contrast), a lane 3: The wild type mouse kidney, a lane 4, and 5:F1 Glyoxalase I transgenic-mouse (lines A and B) kidney, a lane 6: The wild type mouse heart, a lane 7, and 8:F1 Glyoxalase I transgenic-mouse (lines A and B) heart.

[Drawing 3] Drawing showing the carbonyl compound elimination ability under transgenic-mouse organization. The heart origin organization protein extract (last glyoxalase I activity: 0.4 unit/mL) and Homo sapiens glyoxalase of a wild type mouse At

GO or MGO of 100microM, and 37 degrees C, it incubated for 1 hour and HPLC analysis of the heart origin organization protein extract (line A:last glyoxalase I activity: 11.6 unit/mL) of I transgenic mouse was carried out after quinoxaline derivatization. The reaction mixture which does not contain GSH was considered as contrast. A closed bar is a wild type mouse and an opening bar is glyoxalase. The organization of I transgenic mouse is shown. * P< 0.05, **P<0.01.

[Drawing 4] Glyoxalase of a transgenic rat heart tissue origin protein extract Drawing showing I activity.

[Drawing 5] Glyoxalase of a transgenic rat kidney tissue origin protein extract Drawing showing I activity.

[Drawing 6] Glyoxalase of a transgenic rat antinode membrane origin protein extract Drawing showing I activity.

[Drawing 7] Drawing showing GO elimination ability (A) and MGO elimination ability (B) of a transgenic rat heart tissue origin protein extract.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Glyoxalase Drawing showing the structure of the DNA fragment used for the microinjection to the egg which started and was extracted from I installation gene construct.
[Drawing 2] Glyoxalase Drawing showing immuno blot analysis of I gene product. Rain 1:10% mouse erythrocyte suspension (contrast), rain 2:10% human-erythrocyte suspension (contrast), a lane 3: The wild type mouse kidney, a lane 4, and 5:F1 Glyoxalase I transgenic-mouse (lines A and B) kidney, a lane 6: The wild type mouse heart, a lane 7, and 8:F1 Glyoxalase I transgenic-mouse (lines A and B) heart.
[Drawing 3] Drawing showing the carbonyl compound elimination ability under transgenic-mouse organization. The heart origin organization protein extract (last glyoxalase I activity: 0.4 unit/mL) and Homo sapiens glyoxalase of a wild type mouse At GO or MGO of 100microM, and 37 degrees C, it incubated for 1 hour and HPLC analysis of the heart origin organization protein extract (line A:last glyoxalase I activity: 11.6 unit/mL) of I transgenic mouse was carried out after quinoxaline derivatization. The reaction mixture which does not contain GSH was considered as contrast. A closed bar is a wild type mouse and an opening bar is glyoxalase. The organization of I transgenic mouse is shown. * P< 0.05, **P<0.01.
[Drawing 4] Glyoxalase of a transgenic rat heart tissue origin protein extract Drawing showing I activity.
[Drawing 5] Glyoxalase of a transgenic rat kidney tissue origin protein extract Drawing showing I activity.
[Drawing 6] Glyoxalase of a transgenic rat antinode membrane origin protein extract Drawing showing I activity.
[Drawing 7] Drawing showing GO elimination ability (A) and MGO elimination ability (B) of a transgenic rat heart tissue origin protein extract.

[Translation done.]